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RATS AND EVOLUTION

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IN treating a large group of animals from the standpoint of a systematical zoologist, it makes a very great amount of difference whether one does the work in the region inhabited by the animals, or somewhere else with the aid of collections in a museum. A real systematist, of the museum kind, does not come into touch with a number of very real problems which present themselves to field workers, and when he does, he has every inducement to brush them aside with an authoritative gesture, as he is not in a position to valuate their importance. He takes for granted that two similar skins with similar skulls which he receives from the same place, correspond to a multitude of individuals, all with these same characters; that they are a sample of a multitude of animals all exactly alike, and when he finds that animals of such a description have not hitherto been named, he can invent a well-sounding name for the two skins, and publish a description, and henceforth this description of the type specimen and this species name are welded together. If it so happens that an animal is never again collected which corresponds to the published description, the species becomes known as very rare.

There exist conventional rules, which, in the descriptions of species in certain groups, ascribe more value to certain characters than to others. In the systematic classification of rats, the points which are specially noted

in this connection are the shape of certain ridges on the skull, pads on the soles of the hind feet, the relative length of the tail, the length of molar complexes, and the length of the ears.

It is significant to observe, how every field worker who occupies himself specially with rats has his own opinion about the relative importance of these different points for the systematic classification of the animals, and discovers very soon that the work done in museums does not materially help him in his quest.

In 1915 one of us was commissioned by the government of the Netherlands to make a biological and zoological study of the rat population of the Dutch East-Indian colonies, more especially of the island of Java, with the ultimate object to find out what measures could be taken to prevent the exceptionally serious damage to public health and to agriculture caused by rats. Some preliminary work on the subject had been done by medical investigators and by a systematist working with preserved specimens in Holland. The systematic-zoological work in Java was begun some years previously by Maj. G. Ouwens, who is continuing the work after we were obliged, for reasons of personal health, to leave the tropics.

Very soon after arrival we discovered how very little the work done in European museums was to help us out in the field. We are not systematic zoologists, and our reasons for accepting the task lay in the promise the material gave of throwing light on the question of species (in which it has not disappointed us). Therefore the only group of animals with which we have at all deeply concerned ourselves with systematics is the rat, and we would not be prepared to maintain that for other groups the ordinary museum-zoology has so little value in giving a conception of the relationship between species in nature. Still, the study of rats from a semi-economical point of view has certain advantages over purely scientific collecting, as the material studied is very plentiful, and an

extraordinarily great number of keen-eyed persons, public health officials, anxious owners of coffee-plantations, managers of sugar factories, native officials in rice-growing centers, are continually observing the animals, and are more than willing to collect extraordinary large numbers on request. It is not uncommon for any one studying rats to see several hundred animals brought together for him to look over, and one of us has had the occasion to observe a batch of ten thousand rats in one day within the grounds of a sugar factory where between nine and twelve thousand rats were killed daily for several years.

The study of rats has set several authors to speculate as to the nature and the origin of species. Very prominent amongst these is Lloyd (The growth of groups in the animal kingdom). Our conclusions differ materially from those of Lloyd, however. The reason for this difference, we venture to think, lies chiefly in the fact that whereas Lloyd studied dead rats, and speculated upon the origin of his animals, more especially of aberrant types, we have been breeding rats for some six years, and have witnessed the origin of aberrant types. The examples in this paper will be found to be nearly all taken from rats.

When it is found in field work, that two species-names, each given to a skin in a museum drawer, in reality correspond to two real groups in nature, of which they are representative, we may be dealing with one of two different possibilities. It may be that the variability within the first group is not so great that individuals belonging to it fall within the limits of variability of the second group, or it may happen that two different skins in a museum belong to one highly variable group of animals, in which it is difficult to establish dividing lines. If, for instance, two skins with different names in a museum differ considerably in size, it may happen that even the largest animals of the group to which the smallest skin belongs are still very much smaller than the smallest adult individuals of the group which corresponds to the bigger skin. It may happen that two skins are consider-

ably different in a preserved state in respect to some salient character, whereas in nature this very character may be found to be so variable even within a small, closely related family of animals, that it has no value whatever for distinguishing two species. A case in point is the presence or otherwise of flattened hairs, or spines in the coat of rats. On the other hand, it may happen that two species, if once they are dried and preserved in a museum, present no, or, no appreciable, differences, whereas in reality, these two species may be found to differ very definitely biologically. As an illustration we may cite the case of the field-rat and the tree-rat in Java.

The easiest way out of the difficulty is the one taken by a great many zoologists, working through large collections of animals in museums namely, to give a new species-name to every animal which differs markedly from other described species, and which as yet goes without a name.

But if one wants to go deeper into the subject, if one wants to know whether these species of the drawer have their counterpart in as many species in the forest and field, the task becomes more difficult and even hopeless for a great many investigators. As soon as specialists take in hand some group or other, it is very soon obvious that the task of finding out just how many species they are dealing with and how they differ is very much more complicated than it looked when studying the collections in a museum, however well stocked. In treating rat material from a zoological-systematical standpoint, a number of problems confront the investigator from the very outset, and he must try to find his own solutions. Every investigator treats the material in his own way, and where one man makes fifty species, some other man will make two species out of the same material. It is evident, that if the term "species" means anything at all, it must hypothetically be possible to divide the material into a fixed number of species, neither more nor less. The vague way in which the term "species" is applied, must be chiefly

responsible for the unrestricted feeling of personal liberty which systematists undoubtedly have about the way in which they divide a number of dried animals into species. It is for this reason that it here becomes necessary first of all to give our definition of the term "species."

For numerous systematists, a "species" is the description of a skin and a skull deposited in a museum—the type-specimen—and to this species belong all the animals which have just such a skin and skull. Some few botanists are just now trying to reserve the term for a group of animals or plants which have the same genotype, the same set of inherited factors of development. As long as we concern ourselves with autogamous plants, such a definition might pass, we might, at least hypothetically, divide a population of such plants into a number of species and a few hybrid individuals.

It is very obvious that this definition of "species" falls short, as soon as we concern ourselves with animals, or with allogamous plants. In such groups, according to this definition, there would be no species. Even the genotypically purest group of animals would in every instance still be composed of two species, the males and the females, for we now know that the sex difference is caused by a difference in genotype.[1] Therefore, such a definition of the term although very concise and very short, is practically untenable.

When we say: Species are those groups of individuals, which have a common genotype, and which are pure for that genotype, we can most certainly concede to Lotsy that species are not variable,[2] but if we do so, we limit the use of the old word "species" to those groups of plants which really are pure and therefore invariable, so that they can not be changed by selection, natural or artificial.

If we solemnly state that dogs have short twisted tails,

[1] "Mendelian Inheritance of Sex," A. L. Hagedoorn, *Archiv für Entwicklungsmechanik*, 1909.

[2] J. P. Lotsy, *Handelingen van het Natuur en Geneeskundig Congres te Delft*, 1912.

we are perfectly within our rights when we use the term "dog" for bulldogs only. But such a statement brings us no insight in the shape or the length and variability of the tail in the big group of animals which everybody, excepting breeders of bulldogs, knows under the name of "dogs."

We can say: "Carriages have small wire rubber-banded wheels" and if so we are within our rights if we limit the term carriage to baby-carriages, but all such and similar statements of wheat-growers, breeders of bulldogs and manufacturers of baby-carriages, no matter how plausible they may look to the people under consideration, have this one thing in common, that they may not be generalized. Breeders of New Foundland dogs have as much right to reserve the name dog for their animals, and to say that dogs have long bushy tails, as the breeders of bulldogs did, and if we permit the manufacturers of gocarts to reserve the term "carriage" for their product, and if we allow the breeders of autogamous plants to limit the term species to species of wheat and barley and peas, manufacturers of Pullman carriages certainly have the right to state "Carriages are ninety-five feet long and are entered by steps four feet from the ground" and the breeders of sugarbeets or rye, and the zoologists will have the right to state that species are variable.

When we want to make a definition of the term "species" we must make it so that it fits rat-species as well as wheat-species, and in such a way that the geneticians as well as the systematicians can apply it to the things they are wont to call by the name.

We know that all the different genes, all the different inherited factors whose cooperation or non-cooperation to the development of the most diverse organisms produces the hereditary differences among them, are each in themselves invariable. We have called this invariability of the genes Johannsen's law.[3] Only in this way can

[3] A. L. Hagedoorn, "Wetten en Regels in Genetica en Eugenetica," *Handelingen van het Genootschap van Natuur, Genees-en Heelkunde*, 1913.

one explain that those groups of plants, which are so constituted that they become automatically pure in a short number of generation—the autogamous plants—consist in the main of pure and invariable species, which can not be changed by any amount of selection.[4] Selection within a group of plants which descends from one individual, homozygous for all its genes by a continued autofecundation, is ineffective. As we have the name "pure line" for these groups of plants, there is no good reason to limit the use of the term "species" to these groups exclusively.

Liability to change by selection is synonymous with genotypic variability, and this true variability is synonymous with impurity. Those species which do not exist exclusively of individuals which are all mutually identical in respect to all their genes, are variable and therefore liable to change by selection. One single, genotypically pure species as a rule can not give rise to new species. There have become known a few cases[5] of real spontaneous genovariation, mutation, in which every known cause for change in genotype was excluded (one of us has noted three such instances in the mouse); but as in every instance we have been concerned with a dropping out of one gene we can practically leave them out of account here. There exist pure species, but there certainly also exist variable species, species which are certainly liable to change by selection.

In evolution we are certainly concerned with two different sets of processes, on the one hand with the causes of variability, and on the other hand with the processes which limit variability.

Throughout this paper we will call *total potential variability* the quantity of genes which not all the mem-

[4] A. C. Hagedoorn and A. L. Hagedoorn, "Studies on Variation and Selection," *Zeitschr. für Induktive Abstammungs- und Vererbungslehre*, 1914. A. C. Hagedoorn and A. L. Hagedoorn, "Can Selection improve the Quality of a Pure Strain of Plants?" *Journal of the Board of Agriculture*, 1914.

[5] A. L. Hagedoorn, "The Genetic Factors in the Development of the House-mouse," *Zeitschrift für Induktive Abstammungs- und Vererbungslehre*, 1911.

bers of a group have in common, or for which they are not pure (homozygous), and the variability which this impurity makes possible in the descendants.

At least ideally, we can express the potential variability of a group of individuals in a number. There certainly exist species with a total potential variability of zero; these are, for instance, the pure lines of certain autogamous plants, those species for which Lotsy would like to reserve the term species altogether.

We will now try, by the aid of this new term, total potential variability, to give such a definition of the word "species" that it comprises everything which zoologists and botanists, geneticians and systematists, have vaguely meant by it. Our definition is as follows:

A species is a group of individuals which is so constituted genotypically and which is so situated, that it automatically tends to restrict its total potential variability.

Every group of individuals which is closed to the admixture of individuals from without, such as the descendants of an autogamous plant, the dogs or cattle in an exclusive stud, a "Paarungsgenossenschaft" of animals or plants bound by a peculiar habitat, has the tendency to become purer and purer automatically, and to reduce its variability continually. Species originate, given a certain variability of a group of individuals, through all those agencies separately or in combination which bring a group of individuals (not necessarily a small group) into such conditions that the new group has a tendency to become pure for its own genotype. We can not say in general that species are produced by inbreeding, or by isolation, or by a change of habitat, or by colonization, or by selection exclusively. An individual or a group must have a certain amount of potential variability to be able to produce a species, different from the one to which it belongs.

We know now that the genes themselves are invariable. There remain only very few authors who still believe in the variability of the genes. It is therefore necessary to

find out the causes for genovariability. Real mutation, as far as we know, exclusively consists of an occasional loss of a gene without visible cause. Mutation therefore can at the utmost heighten the potential variability by one. De Vries's conception of periods of mutation is at present only of historical interest.

In our opinion, crossing, recombination of genes by mating of individuals of unequal genotype, is to be regarded as the only real cause of variability. There is no good reason to change the opinion of one of us, namely, that there exist three different kinds of variability.[6]

A. Modification, the non-inheritable effect of the non-genetic developmental factors.

B. Real inheritable variation caused by mutation, loss of genes.

C. Real inheritable variation by recombination of genes.

Lotsy has subscribed to our statement (*loc. cit.*) with the exception that he denies the existence of loss-mutations.

We can no more say that species originate by crossing, than that they originate by isolation. New pure lines of autogamous plants, the kind of species for which Lotsy wants to reserve the term, can of course originate in the descendance of one hybrid plant. There is no fundamental difference between evolution in these plants in a state of cultivation and what it must be in nature. But in allogamous organisms, we will only in exceptional cases meet in nature the same course of evolution as in our cages or experimental plots.

Even if crossing in the widest sense is the sole cause of variabilty, we must not suppose that, as a rule, new species come into being in the F_2 or F_3 generation from a cross. If we make a hybrid between species, this hybrid individual will have a total potential variability which is at least as great as the number of genes which were *not* common property of both the forms crossed. If we com-

[6] A. L. Hagedoorn, "Autokatalitical Substances the Determinants for the Inheritable Characters," Roux' serie Vorträge und Aufsätze über Entwicklungsmechanik, Leipzig, 1911.

pose a group of nothing but such hybrid individuals we will get an enormous amount of variability in succeeding generations, and when the group gradually becomes more and more pure for an own genotype this may be a completely new one. A species may have been produced with totally new characters, possibly intermediary between the parent species in some of them. The chance that hybrids of allogamous organisms, even if they are viable and perfectly fertile, will *inter se* produce a new species is exceedingly small in nature. It is much more probable that the process of species formation after crossing is as follows:

There exists a species A, with a restricted potential variability, a set of habits and mode of living all of its own, adapted to a certain environment. As a general rule, individuals of this species A mate exclusively with members of their own species. Once in a while, small groups may split themselves off from the multitude by colonization, and each of these groups will have its own potential variability, and each will gradually become pure for its own genotype, and will be less variable than the multitude.

In the same country there exists a species B, with a slightly different genotype, a different potential variability. Species B is somewhat differently built, somewhat differently coated, compared with A, and therefore fits into a somewhat different environment. As a rule, individuals of the two species do not come into touch. Let us take as examples the grey-bellied *Mus alexandrinum* which lives in houses and on roofs in northern Africa, and the white-bellied *Mus tectorum,* which lives in trees in the same countries. The same holds true for the house-rat and the field-rat in Java, likewise for the house-rat and the tree-rat.

Even if matings between the two species furnish hybrids which are completely fertile, even in localities where two species overlap and are plentiful, the occasional hybrids will be far in the minority compared to individuals pro-

duced by matings between house-rats and house-rats or tree-rats *inter se*. If the occasional hybrids grow up, they will either become house-rats or tree-rats, biologically speaking. In the first case they will mate with individuals of the house-rat population, in the other case with tree-rats. A new group, so situated that its potential variability is bound to be reduced to produce a genotype of its own or a new species, these, a few hybrid rats will certainly not produce. A single mating of a house-rat female with a tree-rat male may be the cause for a heightening of the potential variability of the house-rat population into which the hybrids merge. Eventually this higher potential variability will be reduced again. And reversely, an occasional mating of tree-rat females with house-rat males may be the cause for a greater potential variability of the group of tree-rats to which the females belong.

If it so happens that a few animals colonize out of such a population at the time when the potential variability is still higher than ordinarily, such a colony, which will have a potential variability smaller than that of the multitude, will have a chance of having a range of variability differing from that of the multitude. Such a group may become pure in respect to a somewhat longer tail, a somewhat darker belly or a somewhat greater size, as compared to the population from which it ultimately was derived.

Very good examples of such a process can easily be found by observing the evolution of certain species of dogs or poultry under domestication. For instance, the species Airedale terrier has become variable, and therefore liable to the influence of selection in different directions, because of the fact that hybrids with Dobermann pincher in Germany, and with the Gordon setter in England, have been taken up into the species, the stud not having been closed rigorously, such as the Sloughi stud, or the Jersey cattle stud. But it must not be thought that a new, improved species of Airedale terrier has been

bred from such hybrids *inter se*. The potential variability of the collie was very small, a few years ago. Hybrids with the Russian wolfdog have been taken up into the species. For this reason the variability has been very extensive during a number of years. And at present this variability is reduced again, by selection. In the meantime the species has been changed as a whole, the fashion having changed and having made much of the possibilities afforded by the cross. The collie, which formerly was an intelligent, affectionate dog, with a head shaped like a fox; inclined to be noisy, and to run after everything; with long straight, outstanding hair, and with color ranging between black and tan, and sable, with a variable amount of white, has changed completely. The show collie now is rather a treacherous and surly dog, with a head shaped like that of a llama, silent and lazy, with hair which inclines to be soft and wavy. The color is much more variable and now includes white, slaty, creamy, and generally fade tints.

In chickens, crossing is a common way of "improving" a species, and in all those instances we happen to know, the hybrids were made by using an individual presenting a character which it was thought desirable to fix into the breed, or a certain degree of development of a character, not reached by even the best individuals. Such an individual used for crossing is sometimes a pure-bred animal of another species, but much more often a mongrel of unknown extraction, happening to be somewhat like the breed to be improved, with the exception noted above. It is the practice to breed the hybrids obtained from the cross back to the species, and their offspring again, always selecting those individuals which come nearest to the general conformation of the species, but which have the character to be fixed into the breed. For instance one may want to breed blue Wyandottes. The breeder will then take any blue fowl which happens to look somewhat like a Wyandotte, mate it into a strain of first-class white or black Wyandottes, breed the hybrids back into

his species, selecting from among their blue offspring those which are the most like a good exhibition Wyandotte, and so on, for a number of generations. It is easy to see that in such a case the general potential variability of the whole group is very much increased. It diminishes automatically again, because of the fact that in every generation a few animals produce a great number of offspring. If ten young cockerells of a new species of fowl were habitually derived from ten fathers, the progress in the direction of purification, "fixing" the breed, would be almost nothing. But as ten young males habitually have only one or on the average less than one father, in other words, as only a very small percentage of males in every generation is used for breeding purposes, automatic purification, automatic diminishing of the total potential variability of the group, is very rapid. It is to be noted that in the absence of selection, the group may become pure for almost any conceivable genotype given in the potential variability, the genotypic diversity of the first animals. Therefore any character which has received no or small attention from the breeder may turn out to be different from what it was in the species to be altered into a new breed. It is for this reason, very common to observe that a number of apparently closely related species in the common fowl, or in domestic pigeons, differ, not only in the points which are obvious to every observer, but in other minor points as well, points which need not be in any way correlative to the obvious differences. A few examples. The different species of Leghorn resemble each other very closely, differing to a casual observer in color only. But the comb of black Leghorns is noticeably larger than that in white and brown Leghorns and the ears of the black species are larger than in the brown and the white. The white Leghorn has a lesser tendency to become broody than the buff. The hens lay more eggs than those of the buff or the black breed. The plumage is generally looser and longer in buff Leghorns than in blacks.

In the Wyandotte group of species, the texture of the comb is very different in the blue kind from what it is in the silver Wyandotte. The length of the tail feathers differs very much in the different breeds. The white Wyandotte lays dark brown eggs, the silver Wyandotte lays salmon-colored eggs with minute white spots, the black Wyandotte lays white eggs.

It is very rare for new species in chickens and pigeons to come up to the quality of old established ones, unless the fashion or standard happens to change. The shape and carriage of the tail, and the general shape of the body are very much better in white Fantail pigeons than in the newer black-tailed whites or white-tailed blacks. And the shape of the new blue Wyandotte is not yet what it is in the white and the silver.

We know of only a few instances of new dog or poultry species being bred from hybrids *inter se*. In those cases the breeders had no very definite object in view to start with. This mode of origin of species under domestication is certainly not the common one. Species of cultivated animals are commonly being changed by a very noticeable conscious selection. The variability necessary for improvement is continually kept up, sometimes by deliberate, but mostly by a kind of unintentional crossing, that is to say by admitting exceptionally fine offspring produced by matings of hybrids back to the species, into the registry. On the other hand, automatic purification, automatic reduction of the heightened potential variability, is the necessary outcome of the fact that only very few and very exceptionally "good" males are used as breeders.

Species of tame animals, especially fertile ones as chickens, are easily kept apart so that excessive splitting up into secondary species is possible and even the rule. For instance, in those species in which a certain much sought quality is influenced by the internal secretion of the sex-glands, it is obviously impossible to make a pure strain in which both males and females come up to one

standard. In silver Wyandottes, the standard calls for white feathers, which are bordered by a line of black. Now the males are very much lighter than the females, so that in a pure species, in which the males are correctly marked, the hens are too dark and in a strain in which the hens are good, the males are too light. The only way out of the difficulty has been the establishment of two different species, one which produces correct males and the other which produces exhibition hens. This splitting up of a species into two is very common in chickens. Such pairs of two species are kept as far apart by careful breeders as Wyandottes and Leghorns.

In the natural state, two species, even when hybrids between them are perfectly fertile, and when the individuals exhibit no preference for mating with their kind, may keep apart, if only each group is specially adapted to an own environment, so that the bulk of the animals of each species has no chance of mating with anything but their kind.

Even if there is no adaptation to an environment to keep the multitude of the individuals of a species in their place, two species may keep apart when only the animal's habits keep them from wandering very far. In such a case the borderline, where outposts of both species mix, will present a highly variable population of hybrids of all grades, the variation becoming less and less the more we look for the animals in the exclusive territory of each species. A case in point is, we think, the case of the two woodpeckers cited by William Bateson.[7] When the species differ in only one salient characteristic, the difference between them being in the main due to the presence or absence of only one gene, intermediates must be absent. In such a case the two species may be present in more or less extensive patches, separated from each other by narrow strips of territory having a mixed population. Such seems to be the case of the black and the hooded crows in Europe. Here adaptation plays no rôle apparently.

[7] William Bateson, "Problems of Genetics," Cambridge.

In one territory, two species can coexist only if for some reason matings between individuals of different species are impossible or at least less common than matings between members of the same species, or when the hybrids are sterile.

No matter where we find rats of the Rattus group there are never more than one kind of tree-rat of this group, one house-rat, and one field-rat simultaneously present in one locality, the tree-rat living and foraging in trees, and being exceptionally aggressive, the house-rat living in houses, fearing water, and not afraid of man, the field-rat living even far from cover, scarcely able to climb and too timid to enter inhabited houses. In some regions miniature rats, belonging to the same group, but too small to mate with the bigger species as a rule, inhabit houses and fields, it being very probable that these belong to two species, a small field-rat and a small house-rat.

Such a set of three rat species, a tree-rat, a house-rat and a field-rat, we not only find in Java, but also on Sumatra, the Malay peninsula, British-India and Egypt.

It is our experience that rats of the Rattus group cross with the utmost facility, and produce fertile hybrids. We have come to the conclusion that the majority of house rats remain pure for their own characteristics, even for those which have no value whatever for the adaptation of the species to its surroundings, not because no hybrids are produced with tree-rats or with field-rats, or not because such hybrids when produced are sterile, but for the simple reason that such hybrids are so far in the minority that they disappear into the multitude of house-rats, and that the heightening of the potential variability of the house-rat multitude by such occasional crosses is only local and very transitory. The same is true for the field-rat multitude and the tree-rat population. Crossing produces for each of the three species a heightening of the potential variability, and therefore it is possible that more or less temporarily, there come into being small colonies of somewhat aberrant house-rats or field-rats, in isolated places.

In Solo and Djocjacarta in Java, the great tobacco-growing companies erect enormously big sheds constructed of a very complicated scaffolding of heavy bamboo, with a thick thatched roof made of palm leaves. Such sheds are erected in the midst of the fields, mostly far from native villages. The native laborers leave food about the structure, so that it very soon becomes inhabited by rats. Now the rat population of these drying sheds is always composed of house-rats; field-rats are too timid to live permanently in places where human beings move about so much. But as the sheds are built in isolated places, they do not get their house-rat population as such from neighboring houses. We are convinced that into the composition of such rat populations, field-rats, and hybrids between field-rats and house-rats enter to some extent. This is the explanation of the fact, that very often the rat population of such a shed is found to be composed of an aberrant type of rats. If the population of such a shed becomes very numerous and a native village of some sort springs up in the immediate neighborhood, the aberrant new type may have a swamping influence upon a minority of typical house-rats brought along by the natives, so that the type may become locally common, and temporarily supersede the ordinary house-rat.

We remember Major Ouwens showing us great numbers of white-bellied house-rats, received from a tobacco-growing firm in one of the big centers, Klaten.

When there exists in a certain locality an abundant population of rats of a certain species, immigration of a few rats belonging to the same group but to a different species will have no effect. And of course it will make no difference whether the multitude belongs to the common species and the few immigrants to an aberrant new type, or reversely, as in the case of the rats in the tobacco-sheds.

Ships may occasionally bring rats to Java, from English India, or from Australia or Singapore, but the rat fauna of Java will not be enriched by a new species, as

the result of such an importation. At the utmost, the result will be, that the rat population of the warehouses in the port of entry will become somewhat more variable. It may happen that a single warehouse, empty of goods and rats, will receive a small colony of imported rats with a load of rice and rattan, but on the type of the rats of Java such an occurrence will be of no importance.

A very different thing must happen, when rats from ships come ashore in places where there is as yet no population of rats of that same group, for instance, on newly settled islands. There the imported rat population will gradually become constant, but as often for an own, new set of characters, as for those of one of the species, which originally went into the composition of the ship's population. The rat population of the bigger ships very often is a very peculiar one. It is not uncommon to find a very homogeneous lot of rats on board a ship, for which no corresponding type specimen can be found in any museum. In other instances the population of a ship may be very heterogeneous indeed. The rat population of a ship originates from rats which come aboard with cargo in the most diverse places. By crossing of such animals, all kinds of new types may arise. The rats on board a ship live under very peculiar circumstances. As the animals can not emigrate, their number is absolutely dependent upon the kind of goods stowed in the ship. For a time the circumstances may be so favorable for a multiplication of the animals, that the ship is speedily overrun with rats. Especially is this the case when part of the load affords good hiding places, such as rattan bundles, and if food is abundant, as in a load of copra. On unloading part of the cargo, a famine may result, from the effect of which all the animals, excepting only a very few, may succumb.

The result of such a catastrophe, especially of a series of catastrophes, alternating with periods of plenty, must be, that the population, no matter how variable at one time, must very quickly become pure for a genotype of its own. The occurrence of rats on board of ships is so

common that it must be an exception when a ship transports a number of rats from one port to the other without changing the type.

The rat population of a frequented port can not be taken as typical for the country where the port happens to be situated. It is always easy to find new types of rats for museum collections in cities having much shipping. But it goes without saying, that such animals, with aberrant coat characters, aberrant tail length, aberrant type of skull, perhaps, may not be called species without further ado. It is very possible that at the present moment there exist in Sourabaya twelve still undescribed types of rats, which exist nowhere else on Java. But it is probable that after ten years, thirty-five generations, those types will have all made place for an additional dozen of completely different aberrant types.

Such new types have on Java, which is thickly infested by rats, no chance as house-rats, no chance as field-rats, no chance as tree-rats. A little better is the chance which species have, whose habits of life adapt them to a special environment, where they have little or no competition to fear, or at least only from species which have such a genotype, that they do not mate with the invading species.

Mus norvegicus does not mate with animals of the Rattus group and therefore this species can, without being annihilated, penetrate into a region which is already settled by Rattus rats. We have tried to product hybrids between *Mus rattus* and *Mus norvegicus*. We put eight males and ten females of *Mus rattus* into a large cage, and when we observed the animals mating, we took out the females, and substituted an equal number of *Mus norvegicus* females. The males kept on copulating, but although we saw numerous apparently normal matings taking place, we never got a pregnant female. There is, as we could observe, no real antagonism between *Mus rattus* and *Mus norvegicus*. It is our experience that if we put two Norvegicus rats who do not know each other in a small cage together, there seldom is any serious fight-

ing. As a rule nothing happens in particular when we introduce a Norvegicus to a Rattus rat. But if we put in one cage two Rattus rats which are strangers to each other, they almost always start a fight, and generally it is a matter of life and death.

Mus norvegicus is a real water-rat, sewer-rat, field-rat, and in some parts of Java, where the poor houses have no floor, and where there are many covered sewers, as in Solo and Sourabaya, it becomes a house-rat in a certain sense. But it takes extreme negligence of the inhabitants of a house to make it shelter this rat. It happens that in houses where the bedstead is never moved from its place, and where the space below it is used as a place to dump the garbage, that this rat establishes itself there, excavating numerous large burrows.

It is very remarkable that this rat, which is extremely uniform all through its range in Europe, is very rare in this island, where the geographical distribution shows it to be a recent immigrant, which has come in by way of the big rivers. The skull, the shape of the parietal ridges, the relative size of the bullæ, the relative size of the molars, the relative position of the choane, characters which are very constant in Europe, become very variable here. In size it varies very much, the biggest individuals weighing nearly twice as much as the biggest European animals. The color, which hardly varies in Europe, varies between very light gray agouti to a silvery blackish dark gray, with dark belly there. It looks to us very probable that this great variability may be the result of crossbreeding between this species and species of Gunomys or Bandicota. The variation of the species in Java is certainly towards the characters of these rats, which have a somewhat similar mode of life as *Mus norvegicus*.

As yet it has not been possible to make Bandicoots breed in cages, although we have tried to make them do so in very quiet concrete rooms. Whenever it will be possible to breed these rats it will be very interesting to observe whether *Mus decumanus* will mate with Bandi-

coots, and whether the hybrids are fertile. It seems certainly significant that almost nowhere is the "wirok" population composed of Norvegicus animals as well as Bandicoots. From one locality the people will report and send in gray "wiroks" (Norvegicus), from other localities they will send long-haired black "wiroks" (Bandicoots).

From the standpoint of a systematist, it may look as if it were hardly more than a question of education whether a man is going to follow Hossack and bring all the animals of the Rattus group to one single, variable species, *Mus rattus,* and will look upon the differences between the three main species of this group as uninteresting variations, because he finds all kinds of intermediates in a museum, or whether he is to take the opposite view with certain English museum people, and give a new species name to every couple or trio of rats of a not hitherto described species.

When we start with a drawerful of dried skins, it certainly is a matter of personal taste whether we will distribute the skins over three or ten or twenty smaller drawers, each representing a species. Systematists may quarrel about it, whether a difference in contour of a line on a skull, or a different number of scales on the tail is or is not sufficiently important to make a group deserve a species name, or whether to call it a variety of some other species.

As soon as we have to deal practically with a group of animals like the rats of Java, and have to consider the economic importance of tree-rats to plantations, of house-rats in connection with infections, and of field-rats as regards crops, the museum kind of systematics very often proves insufficient, and we have to begin the work anew in another way.

I remember that one day, among a batch of some ten or eleven thousand rats caught on that day in a sugar plantation, Ketangoengan, there were two with markedly ruddy hue, two with very long tails, three house-rats

(brought by the same boy), one tree-rat and several thousand field-rats. If we suppose a man to prepare a batch of these rats to send them to a zoological museum, this museum would most certainly receive two reddish field-rats, two long-tailed ones, three house-rats, one tree-rat and three normal field-rats. It stands to reason that these dead rats would become five species in the museum, and to anybody looking through the drawer later on, these five species must look equivalent.

By observing all kinds of rats with new characters in the descendance of hybrids, we have become very skeptical indeed in accepting as real existing species those rat species which are represented by two or three skins in a museum, such as, for instance, *Mus Blanfordii,* or *Mus Diardii.*

It is possible that two real species, in the sense that they are real constant types, which remain constant and return to constancy after a cross which heightens their potential variability, not infrequently intercross, the hybrids always disappearing again into the multitude of typical individuals of either species.

The finding of such hybrids has undoubtedly confused the species question very much; on the one hand, several hybrids or sets of hybrids of the first generation, as well as "back crosses" must have been described as species, whereas, on the other hand, some naturalists, through the observation of such intermediate individuals, linking the types of the parental species must have come to the conclusion that they were dealing with only one varying species.

We must never forget that, though certain systematists may think that they can divide a chest of skins, according to their taste or even after profound morphological or biometrical studies, into two or six or sixty species, in reality the boundaries between species in nature are far from arbitrary. And species are really existing genuine groups, with natural, permanent limits.

There do exist very peculiar groups of animals, poly-

morphic species. Whereas polymorphy in autogamous plants really means the existence of a multitude of pure lines, a great many closely related pure species of plants which can easily be seen to originate through occasional crossbreeding, polymorphic species in animals and wild-flowering plants seems to be fundamentally different, in that there is a continual crossbreeding going on without the corresponding automatic purification which we see everywhere. Such species as the ruff and some grouses are always as variable as ever. The street-dog population or the population of non-selected cats in any large town will furnish examples of polymorphic animal species.

Now one of the chief factors in the diminishing of the total potential variability of a group is certainly the fact that a given number of animals in one population are certainly not the descendants of a number of parents of the same magnitude, but of a very much smaller number of parents.

And it is easily conceived how the fact that every female mates with several different males at each conception changes this disparity. This would partly account for the continued existence of polymorphy in the ruff and in the cats and dogs whose breeding is unrestricted, and in the sugar beet.

As to the reality and the limitations, and differences of species, the only way to reach a satisfactory conclusion is breeding them. And the possibility of breeding rats under scientific control is one of the reasons why so much of the experimental probing into the species question is connected with rats.

The Javanese house-rat has a uniformly dark belly, dark feet and a long tail, and a certain ridge on the parietalia which no other Javanese rat possesses. Once in a while a rat is caught in a house or a loembong (rice storehouse) with a short tail, or a somewhat yellow tinge, or with markings on the hind feet, or with a white belly. By a study of these individuals only, it is impossible to find

out whether the species house-rat is really so variable, or whether we are dealing with a new species, or whether they are hybrids having a field-rat or tree-rat father, or descendants, backcrosses from such hybrids. The only way to get light on these questions, which are not only of interest for economics, but for genetics as well, is the making of hybrids. It is very probable that in reality there does not exist anything which corresponds to the dozens of rat species which can be found in all the museum catalogues.

Zoologists and botanists often make short work of the hybrid question, by simply calling all intermediary individuals hybrids. In reality hybrids are very often intermediary, especially when the parent species differ in a great many genes. But very often hybrids show totally new characters which would make them species in the eye of several systematicians.

We mated the small brown agouti house-rat of Java with a large yellow, rather long-haired male, descending from a complicated cross combining *Mus rattus, Mus alexandrinus* and *Mus tectorum*. The hybrids are dark grey, with white belly and orange-ruddy sides, and very much smaller even than house-rats of the same age. Rats like these from a warehouse or from a ship, especially a litter of similar ones as in our case, would certainly have obtained a new species name in a museum. It is not impossible that similar animals with a similar origin are already present in a museum under a new name, as representing a rare species. As long as we had no proof that a new alleged species of rats were not fairly constant under cultivation, and produced a not too variable descendance, we would not accept it as a good species. And even so, we would require to know whether there were anything in its habits of life, or in its relation to other species, which warranted a belief that it would not be swamped in a few generations. For the only thing which distinguishes a species from a variety is the automatic permanency of species as compared to the relative inse-

cure standing of varieties. If all the dwarf mice in a given haystack have white tail tips, because of the fact that the first two mice which happened to find the stack when it was newly made had white tail tips, we can not say that we are dealing with a new species. We have a white-tailed variety of the local species. But if we take a dozen of these mice into our house and succeed in breeding them in cages, we may say that now we have founded a domestic species. This species will continue to exist as long as men will keep dwarf mice in captivity (witness the so-called Irish rats) and long after the stack is broken up, and the few remaining white-tailed mice have been taken up into the normal species. The difference between species and varieties is not determined by the magnitude of the departure from a given type, and it is not a genetical difference. It is a difference in expected permanency. Varieties can become species by migrating into new surroundings, or by a change in surroundings.

It seems more than probable that a great many species in museums are nothing but aberrant types which fall outside the normal variability of an existing species, and have originated by crossing, one or more generations removed.

As we have already said, the only way to find out whether individuals intermediate between existing species have to be looked upon as hybrids, or descendants from hybrids, or as variants of one of the species, is by producing the hybrids and comparing them to the collected material.

It is rather difficult to get rats of the Rattus group to breed in captivity. As we did not succeed in the beginning, we rented a small vaulted room in the ruins of a castle in France, fitted it out with numerous old boxes and baskets, faggots and straw, and turned two females loose in it with one male. There we gave them enough food to last them for a week so as to disturb them as little as possible.

Later on, in Bussum, Holland, we succeeded in breed-

ing the rats in cubical houses of four feet in each direction, made of asbestos slates, and filled with rubbish for the animals to hide in. In the beginning very many animals refused to breed even in these cages, and as the animals were crossbred from the very start, we believe that a sort of very rigorous natural selection must have been the reason for the fact that after a few generations, every couple chosen could be relied upon to breed in asbestos cages, four feet deep and sixteen inches high and wide. These cages were covered with small mesh netting only on one half of the front, and they opened upon a sort of corridor which was nearly completely dark, and could be darkened entirely. In Java some of our rats even bred in small tin cages of the size of kerosene tins. In Buitenzorg the Department of Agriculture has constructed a rat-house from plans furnished by us, composed of a series of concrete rooms, so made, that the animals can be observed from a darkened corridor without knowing it, and a series of masonry tanks with wire covers. This house is used for a biological study of rats, and for experiments in cross-breeding, to determine the status of doubtful material.

It is not necessary to clean the cages very often, if only they are well filled with dry straw and not overpopulated. Disturbing the animals keeps them from breeding freely. It happened that rats of this group bred in open wire-netting cages, but in these cages the danger exists that the mother can not make the nest sufficiently dark and secluded to prevent disturbance by the male. It is our experience, that a young female who has once neglected or destroyed her litter, is almost certainly lost for further work.

As a rule the females do not leave the nest for the first two or three days, or as long as the young are crying. Afterwards, they cover the young in the evening, bury the nest under earth, if they have it, to dig it up again at the end of the night. When the young are three days old, the mother permits young from an earlier litter

to return to the nest, but exclusively the females. Only when the young open the eyes at fourteen to sixteen days, the father is permitted to return into the nest. The young males are kept out of the nest until the young are weaned.

To observe the habits of rats of this group, an endless patience is required, as the animals, which are extremely sensitive to hardly noticeable sounds and movements, habitually are active only at night. If it is possible to darken the room completely, it is possible to observe the animals in the daytime by the light of a small lantern, after rousing them. Weak artificial light seems to make hardly any impression upon rats or mice.

We have seen wild rats, mating and foraging, to continue eating or playing, even when a small lamp was waved to and fro under their very noses, whereas the same animals would be disturbed by the falling of the head of a match. A good plan is to feed caged rats only once a day, at a set hour, to which they accustom themselves very rapidly, as in this way they can be counted upon to be up and doing at a time when it is most convenient to observe them. Even wild-living rats and mice accustom themselves to a fixed hour of feeding. A drawback of the system is that when the supply of food is not more than abundant, delay in feeding of only two hours may cause the death of recently weaned, sometimes even of half-grown rats. The discouragement may be looked upon as being partly the reason of this, for these rats are extremely nervous animals. A shock, a sudden fright, may cause them to lose consciousness for a long time, and fright will often kill them outright.

To be able to observe rats of this group at our ease we tried to tame some of them. Young *Mus decumanus* taken at the time of weaning become tame, or rather stay tame without more trouble. It is impossible to get them tame by taking them at an age of six weeks to two months, when they are wild and apt to bite. Full-grown animals are easier to tame, even if wild caught.

To make *Mus rattus* tame, it is necessary to handle the

nest young before they are a week old, which is possible only if the mother is tame enough to tolerate the disturbance. In the first generations of our work we frequently used tame *Mus norvegicus* females as foster mothers. It is especially necessary to handle the young from the very start at night, when they are very much more active. Infinite patience is required for taming these rats, for if once a young rat jumps from the hand, which easily happens, as they are very nervous, it is impossible afterwards to induce it to remain on the hand. It is possible to get the bigger species absolutely tame, so that they will jump upon the owner's hand when the cage is opened, that they will come to the hand if it is held out, will feed unconcernedly, will let themselves be taken and restrained without resenting it, and that they will not let themselves be disturbed by onlookers even in mating. It is curious to note that they do not seem to know their trainer. A tame rat is tame in respect to all humans. It seems as if taming a rat takes away a good deal of its nervousness, as tame rats are very much quieter even if among themselves, and will breed in smaller cages, and grow fatter than wild ones.

Although we have had a good deal of experience in taming nervous small animals we have never yet succeeded in taming the small house-rat, *Mus concolor*. Even small blind nest young are so nervous that they can not be induced to sit still in the hand without being held. All we could do was to accustom the animals to being restrained without resenting it.

There is a very great difference in the disposition of different species of rats, even in one closely related group such as the Rattus group. The field-rats, Javanese as well as Egyptian, and from Sumatra, behave like *Mus norvegicus*, they are reckless, timid and impulsive. An escaped field-rat can be caught in a moment, because it can be calculated beforehand where the animal will run, namely, along the walls, and thus it can be driven without any trouble into a cage or catching net.

The house-rat, European as well as Javanese, is daring, calculating and relatively little nervous, and on being persecuted, looks out very well for possible hiding places, in which the animal will remain immobile for hours. An escaped house-rat is very often found with the utmost difficulty. Their disposition makes them relatively easy to tame.

The tree-rats, Egyptian as well as Javanese, try to escape from a persecutor by climbing. They are exceptionally aggressive, we have certainly been bitten more times by tree-rats than by all our other rats combined.

In our breeding experiments we used for all rats a card catalogue. Every animal has its own card, on which are noted its number, the numbers of its parents, eventual outline drawings of its markings, and the numbers of the animals it has been mated with, together with the numbers of its young born from these matings. In moving a rat from one cage to the other its duplicate card was moved with it to a receptacle attached to the cage. On the card on file the cage number is noted in pencil. Animals which are dead get a distinctive mark, or their cards are moved to the back of the file. With such cards it is very easy to find out the ascendants and the descendants of every given animal, and it is easy to arrange the card on a big table in the form of a pedigree.

We started our experiments with Rattus rats, by taking over some animals from Dr. Lewis Bonhote. From our experiments, which we are about to describe, it became clear, that crossing is not only the means of recombining the characters of the species crossed, as many English authors have it, but that absolutely new characters may arise by it. This does not mean that new genes came into being; the genes present were recombined in as far as the total potential variability of the hybrids permitted. Only new characters arose, which never showed themselves in the species without crossing.

Dr. Bonhote in England crossed the Egyptian house-rat, *Mus alexandrinus*, a gray agouti rat, with rather

short tail and dark belly, with the Egyptian tree-rat, *Mus tectorum,* a fulvous-agouti smaller rat with a long tail and white underparts, sharply demarcated. The young were all like tectorum. These animals, on being mated *inter se,* gave some dark-bellied young, and from the mating of two "tectorum" young he obtained, together with some dark-bellied and white-bellied agoutis, a few orange yellow rats. At this stage we took over his animals. Through the excessive zeal of the French custom-officers, who feared that the animals might carry malaria(!), they were returned from Dieppe to London, and most of them died on the way, including all the yellow ones. When finally the rats reached us in Verrieres, we obtained only a few white-bellied animals.

White-bellied female no. 13 finally mated in the big room with a black French *Mus rattus* male, after having killed a great many males in cages. The hybrids were black and had very long tails. We lost a good many in transporting the animals from France to Holland.

One of the white-bellied agouti rats obtained, tree-footed number 17, was mated to two black hybrid females, 24 and 25, and with tectorum female no. 19. From the mating of 17 with 24 we obtained twenty young, of which seven were blacks, seven white-bellied agouti (like tectorum), five yellows and a few pearl gray. Among the blacks one had a white tail tip, and one of the white-bellied agoutis had also a white tip to the tail. Three of the gray-bellied agoutis were waltzers. These animals behave exactly like waltzing mice. They run around in small circles with amazing rapidity, and they are unable to climb. But whereas waltzing mice are less viable than their normal litter brothers, the waltzing rats are as vigorous as normals. And whereas waltzing mice are congenitally deaf, our waltzing rats can hear perfectly normally.

From the mating of male 17 with female 25 we obtained seventeen young, seven blacks, of which one waltzer and one white-throated, seven white-bellied agouti, two gray-

bellied agouti, and one agouti with lemon-yellow belly. As females 24 and 25 were litter sisters, with the same parentage, we may be allowed, for the present discussion, to add their young together. There were 37 young, of which 14 were blacks and 17 agoutis (expected 15.5 and 15.5). Of the 37, five were yellow, one pearl gray, two with white tail tip, one white-throated, four waltzers, and one yellow-bellied, all of which are animals with totally new characters.

We can easily explain the origin of the new characters as follows. If both parent species possess a gene, which by its presence or absence makes the difference between a normal and a waltzer, or in other words, if to be normal a rat's germ must at least possess either Y or Z, the hybrids, which are impure for Y as well as for Z, having inherited Y from one and Z from the other parent, will produce one germ-cell in every four, from which both Y and Z are lacking. Therefore such hybrids will produce, when mated among themselves, fifteen normal young and one waltzer in every sixteen. If we expect the same reasoning to hold good for a number of new recessive characters, which are displayed by neither of the parents, so that animals lacking W and X will be yellows, others, lacking U and V, will have white-tipped tails, we should in our case expect to find among our thirty-seven young, two to three (2.312) with the new character in every case. In reality we found yellow five, pearly gray one, white tail tip two, white throat one, waltzers four, yellow belly one, that is 2.33 on the average.

These numbers make it clear that we are not dealing with a sort of period of mutation; it was easy to see that the new types were already given in the genotype of the three species crossed.

Female no. 24 later was mated back to her son no. 95. From this mating we obtained among a number of normal rats, one chocolate and two pearl-gray young. Later we obtained a cinnamon agouti rat, that is to say an animal that probably stands genotypically in a relation to agouti,

as chocolate to black. It is to be remembered that in this group black is dominant to agouti. In all we obtained six wholly new characters from our matings, clearly as the result of the absence of two genes in every instance.

Matings of white-bellied animals with gray-bellied gave either only white-bellied or a minority of gray-bellied in F_2. Gray-bellied rats clearly have a gene less than white-bellied. This is the same result which Morgan obtained in his work with animals of this group. Black was dominant over agouti and clearly there were two kinds of blacks, with or without the gene which makes the difference between white-bellied and gray-bellied agoutis. We never obtained white-bellied black ones. But the blacks with the gene under discussion had a much more deeply black color, very often with a green or a violet sheen. We obtained yellow-bellied yellows, and, just as in the agouti series, white belly was dominant over yellow. Male 28 and female 34, both white-bellied yellows, gave three white-bellied and one yellow-bellied young. Our chocolate and cinnamon rats died on the steamer bringing them to Java. The character white tail tip proved to be recessive. We obtained pearl-gray young and yellows from matings between yellows and pearl grays, but yellows never produced pearl grays. Two agouti animals sometimes produced yellows, but never pearl gray. These were only obtained when one parent was either pearl gray or black. In other words, the factors which produce the difference between black and agouti animals are the same which make the difference between pearl gray and yellow.

Our new rats, waltzers, and animals with new colors, such as they are can not be called species. We can make species out of them by continuing the breed. If we sell a number of animals of one color to rat fanciers, and they get sufficiently enthusiastic over them to provide classes for them at pet-shows, we will be justified in calling such a breed a domestic species.

We saw that in our experiments with rats no new dominant characters originated, unless we want to call

the colored sheen on certain black ones by that name. In every instance there appeared new recessive characters. For every one of them we could see that crossing, recombination of genes, was the cause, not loss-mutation. But it becomes clear that it is very difficult to be sure that apparent cases of loss-mutation are not due to recombination, unless the number of young in the generation in which the novelty appears is rather large. If we mate a species A to a species B, and some yellow or long-haired or albino animals are produced in F_2 we are rather sure that recombination and not loss of genes causes the novel form, even if the number of young is too small to know whether the new character was found in one animal among every four or among sixteen. But if we mate two animals belonging to one single species, and it happens that each possesses a gene which the other lacks, the two genes having equal influence on the development but of such a nature that animals lacking both are albinos, or yellows, the production of a few animals with new recessive character may easily be looked upon as mutation. In such an instance, it will be found that the two animals which produced the heterozygote who gave the aberrant young would be found to be homozygous in respect to the presence of "the" factor. For if we mate an animal having YY to the new form yyzz, all the young will be dark, and none albino. Conversely, if we mate the ZZ parent to the albino, it will also be found to be homozygous, all the young will be colored. In other words, test mating will in certain instances be insufficient proof for the occurrence of a loss-mutation.

In the days when we talked about "unit-characters" and the factors which "determined" unit characters, it was commonly held that crossing in the widest sense, mating of forms with diverse genotype could not count for very much in evolution, as it could only recombine existing characters and not create new ones. We have since learned to look upon the genes as upon things which help with other factors in the development to make an organism develop, and we now know that the action

of genes upon what were called "unit-characters," is a very indirect one. We now know that new characters may certainly come into being through recombination of genes. Recombination may result in the origin of new recessive characters, and this process may look very much like loss-mutation. And crossing may result in the origin of new dominant characters, color in chickens, in rabbits, extra toes in chickens, and this process will look very much like positive mutation, the creation of a gene out of nothing. If we except Œnothera species, dividing the organic world into animals, plants and Œnotheras, for as long as no solution is found for the baffling delayed and abnormal segregation in Œnothera hybrids, we may sum up as follows:

Evolution is the result of a combination of all those causes which heighten variability and which limit it.

The only cause for inheritable variability in multicellular organisms which can be of any account in evolution is mating between individuals of unequal genotype, crossing in the widest sense (Amphimixis).

All those causes which tend to reduce the potential variability of a group of organisms tend to make varieties or species of these groups. Such causes are isolation, migration, adaptation, selection and *especially* the fact that, either periodically or regularly, the number of individuals of one generation is very much smaller than that of the preceding one. This cause of purification of the type, which we see in operation everywhere (think of the numbers of house-flies a year in the last and first generations), operates quite regardless of adaptation or fitness. To this cause working upon variation may be ascribed numerous characteristics for which we can invent no earthly use, and for which nevertheless species are pure.

Whereas species and varieties are realities, systematic division of the organic world into groups of higher magnitude is wholly arbitrary, and may without any doubt be arranged to suit the capacity of museum cupboards.

DIFFERENTIATION BY SEGREGATION AND ENVIRONMENT IN THE DEVELOPING ORGANISM[1]

DR. VERA DANCHAKOFF

BIOLOGICAL investigations in the twentieth century have markedly strengthened the belief in the specificity of different kinds of living matter. Paleontology has shown the existence of organisms which have retained their specificity during millions of years: specific germplasma has carried through ages specific characters. On the other hand discoveries in the world of microorganisms have shown, that even their simplest forms are characterized if not always by specific organization, at least by definite metabolism and other biological qualities which imply a specificity of their constitution.

I shall not discuss the problem of genus specificity. My subject is limited to the specificity of certain tissues and cells found in the organism, the final development of which results in the symbiosis of differently organized tissues. The problem whether the relations of these different tissues is definitely determined by their specificity, or whether there exist in the organism plastic factors which from a homogeneous cell material may mould differently organized products is still unsettled. The solution of this problem would be greatly advanced, if the results of experimental and descriptive histogenesis received due consideration. Though the microscope can indeed not distinguish between various colloidal solutions, it might and does give data of definite biological significance.

Different genera and species show under the microscope a different structure of their building stones—the

[1] From the Anatomical Laboratory of Columbia University. Read before the Section of Biology, New York Academy of Sciences, April 9, 1917.

cells, and most conspicuously in their chromosome-complexes. The specificity manifested by genus and species, whether it is centered in specific proteins of the cytoplasm or in specific molecules of the chromosomes, forms one great chapter of the specificity problem, while the specificity of different tissues and cells is another.

It is often stated that the different tissues and cells of an individual of a given species, all have identical chromosome-complexes. If the chromosomes are considered identical in all the cells of an individual, they can not be regarded as responsible for the specificity of his tissues. They can place no restriction upon a wide range of permutability between the various cells in the organism, can put no restraint upon unlimited regeneration or impede the perpetual proliferation of any type of cells. The assumption of equality of chromosome-complexes in different tissues and of their invariability excludes them from the range of possible carriers of the specificity of tissues and is usually associated with the belief that the specificity of tissues is brought about by segregation of cytoplasmic materials during development. The possibility is also considered, that environment may act as differential factor.

Of these two latter factors the segregation of cytoplasmic materials in the early stage of development leads to the formation of large cell groups (germ-layers, anlages of organs), the differential characters of which are believed to be determined by the presence of definite cytoplasmic materials, transferred to them from the cytoplasm of the ovum. The differentiation brought about by segregation is regarded as irreversible and though the cells of the germ-layers show a great plasticity in their response to different factors, there is a well-marked limitation of their potencies, if compared with the first blastomeres. It is believed, however, that the segregation does not affect the chromosomes and produces merely a differential distribution of the cytoplasmic constituents of the ovum among the resulting cell groups. It has been

recently shown that at least the embryonic mesenchymal cells have an unlimited power of regeneration and therefore can be considered potentially immortal.

In passing I should like to point out that the uninterrupted synthesis of the chromatin during cell proliferation may be secondarily influenced by the differences in the cytoplasm thus acquired. The assumption of invariability on the part of the chromosome-complexes would imply a further assumption of persistence in the cytoplasm at least of some unchanged metabolic processes identical for all cells, to which the synthesis of identical chromatin could be referred.

Differentiation by segregation is a fact proved experimentally and many striking examples of segregation of various cytoplasmic materials during cleavage are found in Wilson's and Conklin's work. As result of segregation a number of cell groups appear. The groups are different, but the cells of each of them are similar. The various cytoplasmic substances distributed to the cell groups are specific, can not be built up by cells, which do not contain them and influence the further development of the cells in a definite manner.

These groups of cells proliferate and differentiate, giving rise to a number of specific organs, tissues and cells. Does a further segregation of definite substances continue at the time of the final specialization of tissues, are the cell potentialities gradually narrowed by further differential distribution of cytoplasmic constituents, and finally rendered univalent and irreversible? Leaving aside the question as to how specific tissues arise from specific anlages, Loeb in his last book, an important and stimulating publication, adopts the view shared also by Stockard, of the specificity of anlages in the organism. *The anlages are,* in Loeb's conception, "*destined to give rise to definite organs.*" He considers "the formation of the various organs of the body, as being due to the development of *specific cells* in definite locations in the organism, *which will grow out into definite organs, no*

matter into which part of the organism they are transplanted." This assumption may apply to a number of developmental processes in the organism, but the statement is only part of the truth. Years of study of the loose mesenchyme and of the differential processes, observed in this tissue, have yielded a few results, which most decidedly do not harmonize with the generalization above quoted.

The loose mesenchyme, which appears in the early stages, is characterized by its ubiquity and by lack of obvious special function, if its mere presence between other organs has not to be considered as function. The mesenchyme is a syncytium of similar cells, the structure and most probably the metabolism of which is little if at all changed, while the cells remain as constituent parts of the syncytium. Influences of local origin which might change the metabolism of some of the cells are inhibited by continuous unimpeded flow and intermixture of substances in the undivided bodies of the cells.

Cells of the loose mesenchyme become isolated from the syncytium in many parts of the organism. This process of isolation is diffuse, in some parts of the organism it affects merely a small number of cells, in others it is displayed with great intensity. Scattered free cells or large groups of them are formed. The cause of such *isolation?* If it is not possible to formulate it in positive terms, at least it can be stated, that *it does not depend upon predestination,* centered in the syncytium itself, since isolation of cells from a mesenchymal syncytium can be greatly intensified experimentally. Large groups of free cells develop in the embryo after certain grafts on its allantois in regions in which normally the cells would retain their syncytial connections.

The free ameboid cells isolated from the mesenchymal syncytium differ from the cells of their maternal basis in many respects. Their metabolism is no longer controlled and regulated by the metabolism of the whole colony of mesenchymal tissue. Isolated they are very active, grow

intensely, frequently divide and their structure undergoes rapidly a series of changes which are not always identical and which transform them into various blood cells. Do these various changes exclusively depend upon the physicochemical constitution of the cells, in other words are they predestined, will each of these cells grow into a definite unit, no matter to what condition it is subjected? Is the group of ameboid, morphologically similar cells freed from the mesenchymal syncytium still formed by a number of species cells, the characteristics of which consist if not in a discernible structure, yet in an inherent necessity to develop along definite lines?

A series of investigations, some of them my own, have pointed to the group of the free ameboid cells as the mother cells of various blood elements. In regions where the isolation is merely occasional, scattered wandering cells arise. In regions where the isolation of free cells is intense, so-called anlages of hematopoietic organs develop. The first stages of development of various hematopoietic organs were found to be much alike and the continuous differentiation of the various blood cells throughout life was shown to have for its starting point a cell, the structure of which is similar to that of the ameboid cell, which arise from the mesenchyme.

Moreover, it was observed that there existed an invariable *association between the development of the mother cell into a definite blood cell and definite environmental conditions,* viz., if left in the spaces amongst mesenchymal cells the free ameboid cell develops into a granuloblast, especially in the vicinity of thin walled vessels; if surrounded by endothelial walls and subjected to intravascular conditions it develops into an erythroblast. This association has been established in the hematopoiesis of birds, reptiles, amphibia and certain fishes. The association between differentiation of the stem cell and environment on account of the regularity with which it was observed suggested to me the idea, that it was more than mere coincidence, and that possibly environment contained the differential fac-

tors, which from a homogeneous cell material moulded different products.

On the basis of descriptive histogenetic studies it seemed plausible to admit that environment can modify isolated cells; that the metabolic processes of the cells are the resultant of their physico-chemical constitution plus physico-chemical conditions of the environment (of course hormons, enzymes and so forth are included in the environment) and do not depend exclusively upon their physico-chemical constitution; that different substances arise in the cell-body (hemoglobin, various specific granules) in polyvalent cells as result of changes, determined by differences in the environment. The existence of cells endowed with various potencies has in consequence been largely admitted. The specificity of the various mature blood cells would thus be brought about by factors extrinsic to the stem cells.

These conclusions are based on facts established by descriptive histogenetic studies. Experimental proofs are beginning to accumulate which soon will leave no doubt of the validity of these conclusions. The existence of polyvalent cells would be proved, if, for example, hemoblasts subjected to various conditions would undergo various differentiation. If stem cells from within the vessels were transferred into the spaces between the mesenchymal cells and here instead of developing into erythroblasts, differentiated into granuloblasts (these experiments are under way) the stem cells within the vessels would be proved to be polyvalent. The same applies to other blood cells. As recently shown, spleenic follicles, the cells of which normally differentiate into small lymphocytes, if grafted on the chick allantois resolve themselves into numerous hemoblasts, which finally undergo a granuloblastic differentiation and give typical granular leucocytes. Thus the results of the histogenetic studies by experimental method entail the recognition in the embryo and in the adult organism of tissues and cells, which have not been fully differentiated and remain polyvalent.

It is the polyvalent cells which are the source of the wide range of regeneration encountered, particularly in the lower animals. It is astonishing to see how readily students of differentiation and specificity reconcile the extensive regeneration observed in many organisms with the belief in the specificity of the anlages of organs. Driesch has shown that gills excised from an Ascidian can regenerate a whole animal with heart, intestine and stolon. If in the particular case the anlages of the gills and the gills themselves were built of specific cells, the results of the experiment would be inconsistent. How could heart, intestine and stolon regenerate from the gills if the cells of the gills were not endowed with various potencies; if specific, they would grow only into the same tissue under all conditions. On what other basis could the experiments of Child's be explained, in which cells of a definite segment in the *Planaria* will regenerate a head or a tail, according to whether it formed the anterior or the posterior part of the piece cut out from the worm? The very fact that different specific structures may be regenerated at the expense of one common source, as, for example, heart and intestine from a gill or erythrocytes, granulocytes and small lymphocytes from hemoblasts, implies the polyvalency of their common source.

It is known, indeed, that environment can educe new qualities in the organism, but they usually subsist only while the specific conditions are present, and are lost if the organism is transferred to another environment. Such changes are not specific. The changes revealed by the freed mesenchymal cells, which result in the formation of mature blood cells, would only then be called specific, if they were retained by generations of their descendants under different conditions. An indifferent hemoblast within the vessels is soon transformed into an erythroblast, which shows in its cytoplasm the first traces of hemoglobin. Is the erythroblast a definitively specific cell, univalent and no longer capable of heteroplastic differentiation in new environment? New environmental condi-

tions for an erythroblast can be found in the organism outside the vessels, where hemoblasts develop into granuloblasts or small lymphocytes. If transplanted outside the vessels, the erythroblasts still developed further into analogous cells, this would mean that the changes which inside the vessels have transformed a polyvalent hemoblast into an erythroblast are irreversible (at least in the organism), that they have narrowed the potencies of the erythroblast in comparison with its mother cell and have rendered it specific, *i. e.* univalent and irreversible in its metabolism. Positive results from such experiment, could they be attained, would be of great value; they would prove that definite factors encountered in the normal organism outside of a cell call forth such changes as would be transmitted by the cell to its daughter cells even if the differential factors had no longer direct influence upon them.

The arrangement of such experiments offers however insuperable difficulties. Hemoblasts or mesenchymal cells can be transplanted, for there are stages in the hematopoiesis of the yolk-sac, in which capillaries are distended exclusively by hemoblasts and at this time they can be transferred into the spaces between the mesenchymal cells. It would be hardly possible to pick out from within the vessels erythroblasts, in which hemoglobin had already begun to develop, but which still were capable of proliferation. Most fortunately the required experiment has been carried out in a series of allantois by nature herself.

The grafting on the allantois which I used in my recent work is often accompanied by an extensive edema in the mesenchyme, which also affected the endothelium of the vessels. At the time of grafting (seventh to eighth day of incubation) the vessels contain numerous young erythroblasts, which after grafting become particularly numerous. The loosening of the vascular wall made it possible for a number of erythroblasts to escape from within the vessels. As a result of these conditions large

groups of cells appeared in the spaces between the mesenchymal cells, which already had begun their erythroblastic differentiation, while within the vessels. These cells, now outside the vessels, proliferate and continue their differentiation into erythroblasts, and their cytoplasm is gradually transformed into or substituted by homogeneous hemoglobinic substance.

The changes undergone by a polyvalent hemoblast within the vessels are thus no longer reversible outside of them. The differentiation determined by environmental conditions has been rendered specific, *i. e.,* univalent and irreversible. The specificity of tissue and cells can not therefore be the result alone of segregation of different cytoplasmic materials during cleavage. The process of segregation, of course, transfers different materials to different cell groups, the presence of which impedes their permutability, but these cell groups are still polyvalent and may, under various conditions, undergo various development.

The relations between these cell groups, the structures, effected by them, the different products of their metabolism, form the external factors of the environment which gradually render the cells of a polyvalent group specific, univalent and irreversible in their potencies. This specificity is transmitted by mother cells to their daughter cells irrespectively of the environmental conditions, to which they are subjected.

A few words concerning the structural changes of the cells during their definitive specialization. Differentiation during cleavage is effected by transmission of different cytoplasmic materials to different cell groups. What kind of changes in the cell structure are induced by the external factors of the environment? Compare the structure of the mature univalent blood cells with that of their mother cells in the stage of a hemoblast. Cytoplasm, structure of the resting nucleus, chromosome-complexes during mitosis, as demonstrated by our microscopical preparation, have undergone such fundamental changes,

as to have required thorough and detailed investigations in order to establish their reciprocal relationship. The size of the cells makes difficult a detailed study of the changes in the chromosomes, and they require further investigation, nevertheless the possibility of distinguishing different types of chromosome-complexes in different cells is not to be overlooked; it is easy to identify, for example, in the thymus entodermal cells, hemoblasts and small lymphocytes, during mitosis by their chromosome-complexes. The assumption of invariability on the part of the chromosome-complexes in the somatic cells requires some qualification. The chromosomes of a cell and the cytoplasm together embody specificity. Changes in both may transform the cell so completely as to deprive it of its faculty of proliferation. Erythrocytes and leucocytes in the blood cell series afford examples of such final modifications which have been gradually determined at least in part by the external factors of the environment.

A METHOD OF NUMBERING PLANTS IN PEDIGREE CULTURES[1]

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ABOUT fifteen years ago, Dr. H. J. Webber (1906, p. 308) introduced into the plant-breeding work of the United States Department of Agriculture a simple and convenient method of pedigree numbering. This method has three essential features: (1) the use of an initial "series number" for each hereditary line or group of lines, the sets of series for different crops being numbered separately; (2) the use of letters for particular parental combinations in a hybrid series; (3) the use of numbers separated by dashes to designate individuals of successive generations.

For example, with cotton, Series 1 might represent selection for longer lint, within the variety Columbia; 1–1, 1–2, etc., would then designate the plants first selected (P_1 generation), and 1–1–5 would designate a plant of the second or F_1 generation.

Similarly, Series 2 might represent a cross between two varieties (for example, Columbia ♀ × Truitt ♂), and Series 3 the reciprocal of this cross. Each combination of "individuals" (plants, branches, or single flowers, as desired) within a hybrid series is represented by a letter, as in 2A, 2D. The F_1 plants are then designated by numbers written after the letters, as in 2A1, 2D6, and the numbers for later generations follow dashes exactly as in a non-hybrid series (for example, 2A1–5). The use of letters thus characterizes hybrid series.

This system of numbering, then, uses simple linear pedi-

[1] Paper No. 40, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station. Riverside, California.

grees for all cases, beginning a new pedigree whenever two lines of descent are combined by crossing.[2] It is adapted to self-fertile organisms, and its convenience and usefulness obviously increase with the frequency of selfing. Pearl's (1915) "system of recording types of mating," on the other hand, is especially adapted to diœcious and self-sterile organisms, and in general its usefulness for recording or presenting data increases with the frequency of crossing.

Several systems similar to Webber's have been described in recent genetical papers; these schemes usually differ mainly in the method of indicating the "series" or "families." In Jennings' (1916, p. 415) system for asexual reproduction with the animal Difflugia, for instance, each "family" is a pure line descended from one selected individual; each pedigree number, consequently, begins with a simple number assigned to the P_1 individual. Here we have the simplest possible form of pedigree numbering. With the higher plants, however, we need some special provision for cases of crossing, and also some method of distinguishing asexually produced individuals from those produced sexually.

Belling (1914, pp. 309–10),[3] for Stizolobium crosses, uses for each series the initials of the common names of the parent species (*e. g.*, VL for velvet bean ♀ × Lyon bean ♂); this is practicable because the crosses involve only a small number of named forms, themselves appearing practically constant. For the same reasons, the P_1 plants, and usually the F_1 also, are not individually indicated in published pedigrees; VL3 is an F_1 plant, and VL3–7 or VL–7 an F_2 plant. The obvious result of this procedure is a gain in both clearness and brevity of presentation.

Hayes and East (1915, p. 3) use for maize crosses a scheme that resembles Belling's in its direct indication of the parentage of hybrids. "The various races were given

[2] Except in cases where vicinism is undiscovered or ignored.

[3] Or see Fla. Agr. Exp. Sta. Report for year 1913, 1914 or 1915.

different numbers as No. 10 flour corn and No. 5 flint corn." Then the F_1 hybrids would be designated, for example: (10 × 5)–1, (10 × 5)–2, (5 × 10)–1, etc. If, however, the individual P_1 plants are regularly indicated in the series numbers, these numbers tend to become unwieldy, particularly if the P_1 plants themselves have somewhat lengthy pedigrees. In cases where this objection does not apply, and where Belling's method is inadequate, Hayes and East's scheme has some advantage in the immediate significance of the series numbers of hybrids.

Of the systems described, Webber's seems most generally applicable to work with the higher plants, though the others may be somewhat more convenient in certain cases. I wish to present several additions to that system, designed in part to provide for somatic variation and polyembryony.

First, capital letters may be used, as is often done, for various special purposes. For instance, letters are sometimes added to distinguish particular types; Shull (1908, p. 60), in describing his system of pedigree records, restricts letters (aside from Roman numerals) to this use, and Hayes and East (1915, p. 4) use them to designate floury and flint-like types of maize kernel. When temporary lot numbers are used, "L" prefixed to these numbers will distinguish them from any others. Again, Arabic numerals preceded by "R" seem more convenient than Roman numerals as used by Shull (1908, p. 60) to designate rows in field cultures—giving, for instance, R1, 3 (row 1, plant 3) for I, 3, and R28, 26 for XXVIII, 26. I would suggest that capital letters, and capitals only, be employed for such miscellaneous purposes.

Second, small letters may be used for the indication of parts of individuals, whether the parts remain attached or are separated in vegetative propagation.[4] Then, "–51a" in a Citrus pedigree will indicate a particular part of tree 51 in a certain generation of that series—

[4] This is to replace a method of indicating parts of individuals by means of fractions.

perhaps a part permanently marked, and noted in the records, because of some somatic mutation. In the same way, a small letter affixed to any individual designation of a plant not in a pedigree culture, such as, for instance, the number of a tree in a field experiment, may be used to indicate a particular branch. A small letter used alone will indicate a "generation" of vegetative propagation; for example, in the case mentioned above, "–51a–a" and "–51a–b" would be trees budded from the mutant branch "–51a."[5]

Third, small letters affixed to the series number may be used to designate individuals of the first (or P_1) generation of any series, *whether hybrid or not.*[6] The definition of *individual* would necessarily be determined and recorded for each type of work; where desired, the "individual" may be a branch, or even a single bud or fruit. Thus, in my work with Citrus, a letter is assigned to each self-pollinated branch giving seedlings. For example, series 28 consists of descendants of selfed Valencia orange; "28a," then, indicates one bagged branch of Valencia, "28b" another branch, etc. The F_1 progeny will be 28a–11, 28a–12, 28b–32, etc.

For a hybrid series, on this plan, two letters are used to designate the two parents, the letters being independent of those used in the corresponding non-hybrid series. For example, my Citrus series 27 represents Valencia orange ♀ × Imperial pomelo ♂, and "27aa" designates a cross between two particular branches. 27aa–21, then, will be an F_1 hybrid.

Similarly, in my work with Matthiola mutants, series 23 indicates the cross Snowflake ("normal") type ♀ × Slender type ♂. "23ea" designates the combination of

[5] The complete pedigree numbers might be 28a–32–51a–a and 28a–32–51a–b (see below).

[6] In cases where the initial parents of a series are selected from larger groups requiring designation of individuals, the plants of these larger groups may be given temporary numbers. For example, my Raphanus series 14 began with plants from one lot of commercial seed of one variety, numbered 14, 1; 14, 2; 14, 3; etc. In the next generation, 14, 3 became 14a, and 14, 16 became 14b.

two particular individual *plants,* not of two particular *branches* as with Citrus.

Thus with Matthiola a series starts with the crossing, in a given direction, of two apparently uniform *seed-reproduced types,* while with Citrus a series starts with the crossing of two *clons.* In another case it might be desirable to ignore some evident genetic variation in features not being studied, and to base the delimitation of the series on genetic differences of seed-reproduced P_1 individuals in some particular feature under consideration (*e. g.,* in color characters).

Fourth, in cases where polyembryony is to be expected nine numbers (1 to 9), instead of one, may be given, so far as needed, to the product of each seed. Thus, with the Citrus cross mentioned above, the first seed will be assigned the numbers 27aa–11 to 27aa–19, the second seed the numbers 27aa–21 to 27aa–29, etc.[7] The actual plants from the first three seeds are therefore designated as follows:

Seed No. 1 (2 plants), 27aa–11 and 27aa–12.
Seed No. 2 (2 plants), 27aa–21 and 27aa–22.
Seed No. 3 (1 plant), 27aa–31.

If, then, two trees are budded from tree 27aa–31, these will be 27aa–31–a and 27aa–31–b.

It may be objected that this treatment of polyembryony gives misleading numbers to plants from adventitious embryos. It may, however, be impossible in many cases to differentiate positively these asexually produced progeny before maturity, if at all without progeny tests, and the probable inapplicability of some of the numbers must be kept in mind. Where, as with crosses of *Citrus trifoliata* with Eucitrus species, the strictly maternal individuals can be separated at an early stage, they can be discarded; or, if such plants are to be kept, and their exact origin indicated, letters may be used, thus:

43aa–11 (true hybrid).
43aa–1a (for 43aa–12) } (plants from adventitious or
43aa–1b (for 43aa–13) } asexually produced embryos).

[7] The seeds themselves might be called 27aa–10, 27aa–20, etc.

By thus inserting letters in the hybrid number, to designate the second and third plants, we indicate the vegetative origin of these plants, and also their quasi-sib relation to the true hybrid.

Open pollination, or lack of protection of flowers in general, can be indicated in the numbers, if this seems desirable, by underscoring the proper letter or number,—the use of two letters with the series number for hybrids making it possible to do this with the pollen parent as well as with the seed parent.

An incidental advantage over the original plan results from the use of letters with the series number in the way here indicated. This consists in the fact that the numbers for hybrid and non-hybrid series are thus made symmetrical in relation to the number of generations involved. As an illustration we may take the case of the Matthiola cross mentioned above. This cross was made in 1914, and the hybrid seeds were planted, together with seeds from selfed parents of the same types, in 1915 and 1916. Corresponding to the F_1 hybrids 23ea–1, etc., we have the F_1 selfed progeny 19a–1, etc., from a Snowflake parent, and 25c–1, etc., from a Slender parent, while by the original method these three numbers might be 23G1, 19–1–1, and 25–3–1.

As will readily be seen, this scheme is elastic; if the five main features stated above are adopted, different workers may add, modify, or omit various details, and still use numbers intelligible to each other with little or no special explanation. Further, the method of designating series and initial individuals is essentially independent of the other features suggested, so that any of the latter features may be adopted without the former.

All the schemes so far discussed give *cumulative* numbers, which include the whole pedigree from the P_1 individuals down. Shull (1908, pp. 59–64) has described a non-cumulative system, in which individuals have only temporary numbers (in the field depending on row and position in row) until selected as parents. Each parent

is given the number of the notebook page on which its progeny are to be described, preceded by two figures indicating the year in which those progeny are grown; *e. g.*, 06230 is a parent whose progeny are grown in 1906 and described on p. 230 of the 1906 notebook. Only parental and grandparental numbers (*e. g.*, 0557.230 for the case just mentioned) are shown at the head of each notebook page, but these numbers permit ready reconstruction of pedigrees from the pages indicated. The labels bear only the parental number.

In Shull's scheme, then, there is no cumulation beyond the second generation, even in the numbers as written in the notebook. The numbers would include 4 to 8 figures (*e. g.*, 083.7, or 05157.230) in the notebook and 3 to 5 figures (*e. g.*, 097, or 06230) on labels. In the scheme here suggested there is a continuous cumulation; a plant of the second or F_1 generation is usually represented by 3 to 7 letters and figures (*e. g.*, 5b–8, or 32ba–251), and after from 2 to 4 more generations the numbers begin to be decidedly unwieldy. The greater inconvenience and danger of error in copying these larger numbers would seem, however, to be largely offset by the growing familiarity to the worker of the earlier part of a pedigree, and by the identity of the temporary and permanent designations of individuals. Further, the page-to-family feature would be inconvenient in some cases, where several or many actual pages are given to one progeny lot and so would require the same number.

With a cumulative system, the simple device of using temporary yearly lot numbers on individual labels will obviate the necessity of writing a long pedigree number for each plant. If the full number is written in the notebook and on each lot label, together with the lot number (*e. g.*, L1 = 16aa–6–3–44–18–3), and the latter alone on the individual labels if these are used, much work can be saved. In an extreme case, the parents belonging to a given series which are included in a given culture can be

arbitrarily made the initial (P_1) individuals of a new series.

The system here presented has been used for two or three seasons' work with the two types of hybridization mentioned above, with Raphanus hybridization beginning with highly heterozygous material, and with pure-line breeding of the tepary bean (Phaseolus). It appears, so far, to be adequate and generally satisfactory for all these types of work.

SUMMARY

This paper describes a system of pedigree numbering adapted to various types of genetical work with the higher plants.

Other similar systems secure greater brevity or clearness in certain cases, but are usually of less general applicability; the main differences relate to the designation of series and their initial individuals.

This system provides for polyembryony and somatic variation, and permits of the addition of various other refinements in cases where they may be needed. The basic scheme is perhaps as simple and convenient as is consistent with use for all purposes without change in essential features.

In this and similar systems, the numbers are cumulative; Shull's non-cumulative system has both advantages and disadvantages in comparison.

To summarize the most essential features of the proposed method: (1) the series for a given plant (genus or species) are numbered consecutively; (2) the initial "individuals," as defined in the records, are denoted by small letters affixed to the series numbers; (3) in each following generation the individuals are numbered (if sexually produced) or lettered (if asexually produced), an affixed letter indicating a particular part of an individual; (4) reproduction or propagation is always indicated by a

dash;[8] (5) capital letters are employed for miscellaneous special uses.

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[8] Of course, a period may be used, as is done by Jennings (1916) with Difflugia, with a saving of space but possibly a greater liability to error in copying.

SYNCHRONISM AND SYNCHRONIC RHYTHM IN THE BEHAVIOR OF CERTAIN CREATURES

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IT is a matter of common experience to observe instances of synchronous behavior and expression among creatures. In such instances an entire group of creatures may react simultaneously to the same external stimulus. A flock of birds will arise from the ground and dash away at the first signal of danger, or a school of fish will swerve as a unit from a stick pushed toward them. In the same way certain frogs in a pool may be started into a brief, explosive chorus of simultaneous croaking by the notes of a single individual. After a brief period during which all have expressed themselves, silence ensues until the next singing-reflex is unlocked by the croaking of another individual. In New England I have heard the wood frog, or so-called clucking frog (*Rana sylvatica*) give rise to just such outbursts of simultaneous clucking, started either by the frogs themselves or by my giving an imitation of their notes during an interval of silence. This habit of singing in concert is not unusual among certain species of frogs, and is mentioned by D. D. Cunningham (1903) in his excellent book, "Some Indian Friends and Acquaintances." He says:

> Such utterances recur several times in succession; a short pause follows and then the conversation begins again. The curious thing is that all the performers seated in one patch of swamp should have such a tendency to synchronous action that periods of total silence alternate with those of general uproar. The phenomenon is parallel to that of the synchronous luminosity that sometimes occurs so markedly in groups of fireflies.

In these instances synchronism is maintained at intervals, but there is no regular rhythmic expression within the group itself. Each frog croaks in its own way, until a perfect babel of noise is produced.

Similar synchronous outbursts of sound also occur among the musical insects. The tendency to respond to the notes of their kind is very strong, and a single singer

may be followed by thousands. Although the seventeen-year cicada rarely sings at night, Hopkins[1] noted a most remarkable nocturnal concert which began with a single singer. He says:

> I was fortunate enough to hear the starting of one of these concerts on a clear, moonlight night in June. One male in an apple tree near the house suddenly called out as if disturbed or frightened. His neighbors in the same tree were thus apparently awakened. One started the familiar song note, which was at once taken up by numbers of other males, and, like the waves from a pebble dropped into still water, the music rapidly spread until it reached the edge of the thick woods, where it was taken up by thousands of singers, and the concert was in as full blast as if it had been the previous day. This continued a few minutes, until all had apparently taken part and the song had reached its highest pitch, when it began to gradually subside, and in a short time silence again prevailed.

In his book, "Bolivia" (1914), Paul Walle (p. 268) speaks of the sudden outbursts of noise in the tropical wilderness as follows:

> As the darkness grows deeper, the silence of the forest is broken by the sound of flight, by sudden rustlings, or by a strident shriek. Immediately the creatures of the forest raise their various cries, the uproar lasts for a moment, and all relapses into a silence in which one still hears, more or less sensibly, the murmur of a million insects.

In such instances where a group of creatures respond simultaneously to the same initial stimulus, we have the simplest case of synchronic behavior. I once witnessed an instance of a similar synchronic behavior in the movements of a colony of plant lice which thickly covered the tip of a twig. While I was watching them, a tiny, parasitic wasp suddenly approached and hovered over the colony preparatory to attacking them. The plant lice became aware of its presence and in an instant the entire colony raised the hind portion of their bodies simultaneously into the air at an angle of about 45° and began waving their hind legs about. This behavior was probably more or less a protective response to the primary stimulus afforded by the presence of the wasp. It is also possible that the reaction, once started in a few individ-

[1] "The Periodical Cicada," by C. L. Marlatt, Bull. No. 14, Div. of Entomology, U. S. Dept. of Agr., 1898, p. 58.

uals, would tend to be transmitted to the entire colony by the mere contact of their bodies.

The same sort of synchronous movement may sometimes be noted in the reactions of the leaves and leaflets of the sensitive plant (*Mimosa pudica*). A sudden shock may cause every leaf of the plant to react synchronously. Sometimes, when the stimulus is applied only to one or two pairs of the basal leaflets, the closing of these and their contact with those beneath them produce a progressive closing movement in all the pairs of leaflets until the terminal ones are reached. In this instance we have a progressive transmission of stimuli quite similar to the transmission of certain automatic reactions which sometimes take place in groups of insects, birds, or animals.

In other instances the reaction to a certain stimulus may involve several similar, instinctive movements producing the simplest form of synchronic rhythm. One interesting instance of this sort of synchronous action has been adduced by H. H. Newman, in *Science*, N. S., for January 12, 1917. Newman found that an enormous colony of "harvestmen," of the genus *Liobunum*, resting beneath an overhanging rock, when disturbed began a rhythmic body movement, raising their bodies up and down at a rate of about three times a second, for a brief period. If the stimulus was set up in a few individuals of the colony, the synchronous body movements spread rapidly over the entire colony. After a time it was found that the reactions became weaker and finally ceased. In this instance it appears that there was a secondary transmission of the stimuli from individual to individual by means of their closely interlocked legs. The writer has noted a similar behavior among the individuals of certain caterpillars which were arranged close together on a twig. These caterpillars, when resting, had the habit of keeping the anterior portion of their bodies raised in the air. If the colony was disturbed, each individual began a synchronous swinging of the free portion of the body from side to side, violently, for a brief period. The primary stimulus could affect all the caterpillars of a group, or a secondary trans-

mission could take place down the twig. Although the swinging was not always in the same direction, a remarkable degree of synchronic movement was brought about.

In those instances where the rhythmic expression of each individual, when set up, continues for long periods of time, as in the case of certain crickets, we have the development of a more complex synchronic rhythm having its origin in the instinctive habit of response, and probably also built up and maintained by the unconscious influences of the regular sounds of the crickets upon one another.

I do not feel the slightest hesitancy in affirming that certain crickets which have the intermittent habit of chirping may build up and maintain a synchronic rhythm under favorable conditions. I refer to the well-known snowy tree cricket (*Œcanthus niveus*), whose synchronous music has been noted and described by many well-known observers and naturalists in this country. I have many times heard the remarkable synchronous chirping of these crickets in New England, and under exceptionally favorable conditions the synchronism has been so marked that waves of solemn, rhythmical music have been produced for long periods of time. This rhythmic music was spoken of as a "slumbrous breathing" by Thoreau. Hawthorne called it an "audible stillness" which, "if moonlight could be heard, it would sound like that." Burroughs called it a "rhythmic beat." McNeill has said of the music of this cricket:

> It is heard only at night and occasionally on cloudy days, but in the latter case it is only an isolated song, and never the full chorus of the night song produced by many wings whose vibrations, in exact unison, produce that characteristic "rhythmic beat," as Burroughs has happily phrased it.

Dolbear, in the AMERICAN NATURALIST for November, 1897, refers to this cricket when he says:

> At night, when great numbers are chirping, the regularity is astonishing, for one may hear all the crickets in a field chirping synchronously, keeping time as if led by the wand of a conductor.

Although the facts may have been slightly overdrawn when Dolbear stated that *all* the crickets in a field were

chirping in unison, Shull was not justified in concluding that the synchronism observed by Dolbear was merely an illusion. If such were the case, it is surprising that so many other excellent observers have also been misled by the same illusion in reporting the music of the snowy tree cricket. For many years I have made a very close study of the stridulations of insects, yet I have noted this synchronous chirping in but one other species of those crickets which possess the intermittent habit of trilling. One may frequently hear great numbers of the common field cricket (*Gryllus pennsylvanicus*) chirping in the fields, but these crickets never show the least tendency to chirp in unison as do the snowy tree crickets. The jumping tree cricket (*Orocharis saltator*) is also an intermittent triller and may sometimes be heard chirping in great numbers in certain copses, yet the "illusion" of synchronous trilling one somehow never experiences. Likewise, there appears to be no tendency whatever for the ground crickets, *Miogryllus saussurei, Nemobius fasciatus,* or *Nemobius ambitiosus,* to chirp in unison. Many other locusts and katydids, such as *Conocephalus ensiger, Conocephalus exiliscanornus,* and the common katydid (*Cyrtophyllus perspicillatus*) produce regular, intermittent notes and stridulate in well-defined colonies, yet so far as I have observed the individuals of a species never show the least tendency to stridulate in unison.

In the south, however, I have heard the tiny tree crickets (*Cyrtoxipha columbiana*) chirping in unison with remarkable precision, producing waves of shrill, rhythmic sound, as in the case of the snowy tree cricket. In this instance great numbers of these crickets were located in the branches of a large evergreen holly tree. The shrill notes of this little cricket are delivered with great regularity, as are the low, solemn chirping notes of the snowy tree cricket. This regularity in the delivery of the chirping of these two crickets is especially striking when compared with the musical efforts of other chirping crickets, such as *Œcanthus angustipennis, Orocharis saltator,* or *Gryllus pennsylvanicus.* This sustained regularity in the rate of chirping of the snowy tree cricket has been noted by

various observers. On the supposition that the rate of chirping of these crickets is entirely a function of temperature, it has even been considered that the crickets may serve as an accurate thermometer. As temperature indicators, however, the crickets can not always be relied upon under all conditions, since wind, humidity, and electrical conditions of the atmosphere preceding thunderstorms, also appear to influence the activities of these insects.

It is now a question as to whether these crickets perceive the rhythm which is so pronounced in the regular sequence of their chirpings. I believe they must, for it is quite evident that they hear and respond to the peculiar rhythmical chirpings of their kind, which have become the common language of the species. If they are able to recognize the notes of their kind, it is reasonable to believe that the rhythmic character, as well as pitch, manner of delivery, and even more subtle tonal differences enter into the recognition.

The rhythmic chirping in unison which oftentimes becomes such a pronounced feature in the music of these crickets takes place only in the evening and appears to depend upon the nice adjustment of certain nocturnal atmospheric relations—moonlight, temperature, humidity, and stillness of the air. Early in the evening, perhaps, a single cricket begins its stridulations which stimulate others to respond, and by degrees the great chirping chorus is augmented. There may be no noticeable synchronism in the chirping at first, but if conditions are favorable, the crickets gradually build up a synchronic rhythm until waves of solemn music are produced by a certain colony.

There seems to be a marked tendency for the individuals of each colony to adopt the rhythmic beat of their particular colony, so that not infrequently a neighboring colony may establish an antiphonal rhythm, with the result that waves of quavering sound swing backward and forward between two neighboring colonies.

How is this synchronal chirping built up and maintained? It is probable that the instinctive habit of re-

sponse tends to bring the chirping of many individuals into a regular synchronism, but I feel, also, that the crickets are somehow unconsciously influenced by one another in their chirpings so that they tend gradually to build up a common synchronic rhythm as the night advances. Once a synchronic rhythm is established, I am inclined to feel that it would be natural for those individuals which began chirping, at first asynchronously, to chirp, sooner or later, in unison with their fellows.

From the tone of certain discussions which have taken place in *Science* for the past several months, it is clearly indicated that the synchronous flashing of fireflies as a reality is somewhat doubted. There is no reason to consider such synchronism "contrary to all natural laws," as Laurent as recently stated in *Science* for January 12, 1917. In the tropics it appears that great numbers of fireflies frequently establish themselves in the crowns of certain trees, and I am not at all ready to deny that under certain conditions such colonies may not flash synchronously, as reported by Cunningham (*loc. cit.*), Shelford, and others. It is unfortunate that in most instances these reports are accompanied by very meager details as to the general behavior of the fireflies in these arboreal colonies. Cuthbert Collingwood, however, in his book, "Rambles of a Naturalist," (1868), pp. 254–255, has given a very careful account of his observations of the synchronous flashing of fireflies, as follows: "At Singapore, and also at Labuan, the little luminous beetle commonly known as the firefly (*Lampyris Sp. Ign.*) is common. When flying singly it shines with an intermittent light which alternates with darkness; but on fine evenings and in favorable (*i. e.,* damp and swampy) locations, they present a very remarkable appearance. Clustered in the foliage of the trees, instead of keeping up an irregular twinkle, every individual shines simultaneously at regular intervals, as though by common impulse; so that their light pulsates, as it were, and the tree is for one moment illuminated by a hundred brilliant points and the next is in almost total darkness. The intervals have about the duration of a second, and during the intermission only one or two re-

main illuminated. To all appearances they are not on the wing at the time but settled upon the tree; for I was able to recognize certain points of light which I especially noticed, and which remained in the same situation with each successive flash. When I disturbed them under such circumstances they flew about at random, each giving out a more rapidly intermittent light. At Labuan, however, I have frequently seen them shine with a steady light as they flew along looking like little, flying stars of the second, or even first, magnitude." If there are periods of repose or inactivity during which the insects cease their flashing, it is easy to see how a part of the colony or even all the insects could react simultaneously to the same visual stimulus, such as the sudden flashing of a single individual. If it is the habit of the insects, before again becoming quiescent, to flash several times in succession, following an appropriate stimulus, it is very easy to see how distinct synchronous flashes of light would now and then illuminate a portion of the tree, or even the entire tree. Such synchronic rhythm in the flashing of fireflies would be similar in every way to those instinctive, automatic body movements observed by Newman in the case of the "harvesters," *Liobunum*—the swinging of the pendulum, so to speak, two, three, or more times, as the case may be, following the initial stimulus. As Newman well suggests, it is possible that a transmission of stimuli could even build up and maintain for some time a synchronous flashing in colonies of fireflies in a tree or field. This flashing in unison would parallel the synchronous trilling of the snowy tree crickets, and would not necessarily violate known natural laws governing the instinctive synchronic activities of various creatures.

By selecting two individuals which were evidently chirping in unison, Shull[2] attempted to study the reality of synchronous chirping in a colony of snowy tree crickets by statistical methods. No greater statistical fallacy

[2] Shull, A. F., "The Stridulation of the Snowy Tree-cricket (*Œcanthus niveus*)," *Canadian Entomologist*, Vol. 39, 1907.

could be adduced, however, than to attempt to determine the reality of synchronism in a colony of chirping tree-crickets from statistical results obtained for only two crickets of the colony. That synchronous chirping may be a reality, it is not necessary that 100 per cent. of the individuals of a colony chirp exactly in unison. A few crickets chirping asynchronously would not necessarily prove that a synchronism did not exist. As a matter of fact, some individuals always chirp asynchronously, even when the synchronic rhythm is most pronounced. Taking any two crickets from such a colony, the results would depend entirely upon which two crickets were chosen, and either perfect synchronism or absolutte lack of synchronism, would be established.

It is obviously impossible to subject all the individuals of a colony of chirping tree-crickets or a group of flashing fireflies to statistical analysis. Because this is impossible, however, one is not justified in concluding that judgment based upon careful observation is of no value in determining the reality of synchronism. If one hundred men were marching down a street and 75 were in step while 25 were not, judgment alone would establish the fact that a synchronic rhythm existed. This would be quite as true for the chirping of tree-crickets or the flashing of fireflies. As a matter of fact some of the most marvellous discriminations depend upon niceties of judgment alone, and no amount of statistical data would simplify the matter.

I can not yet agree with those who are inclined to believe that the snowy tree-crickets never chirp synchronously, and that it is impossible for fireflies to flash synchronously, especially certain tropical species of fireflies. I will agree, however, that in practically all instances of synchronic rhythm there seems to be no evidence of conscious, intentional imitation, but merely instinctive, reflex, or automatic reactions to certain stimuli, similar in many respects to the unconscious reactions of the leaves and leaflets of the sensitive plant.

SHORTER ARTICLES AND DISCUSSION

SOLID MEDIA FOR REARING DROSOPHILA

BAUMBERGER and Glaser (1) recently described a method of raising the banana fly on transparent solid media, thus enabling the investigator to observe more accurately the rate of growth and metamorphosis and the larval habits of this insect. The medium was made as follows:

> Five or six bananas were mashed up in 500 c.c. of water. This was allowed to infuse on ice over night, after which the liquid was passed through cheese cloth. Powdered agar-agar was then added in the proportion of 1½ gm. to 100 c.c. of the banana infusion. This was then heated until the agar had dissolved. The liquid was then filtered through a thin layer of absorbent cotton into test tubes. The tubes were then plugged, sterilized and slanted in the customary manner.

As pointed out in the above article, one of us (2) had observed that the bacterial growths which always develop on this medium "do not seem to harm the larvæ" and the mold which sometimes appears "is usually destroyed by the larvæ just as soon as they hatch." This question was further investigated and it was found (2) that the prinicpal food of *Drosophila* is yeast and the flies can not develop on banana which is kept free from microorganisms.[1] Delcourt and Guyenot (3) had previously (unknown to the author) published similar conclusions and Loeb and Northrop (4) had confirmed them shortly before the author's report (2) was sent to press.

It is therefore very well known that the food of *Drosophila* is yeast and the prime necessity of any medium for rearing this fly must be either abundant food for yeasts to grow upon or the presence of large numbers of yeast cells. If a medium is made of sterile compressed yeast and agar-agar[2] it serves as a perfect food for flies which have been freed from microorganisms; however, if living yeasts develop young larvæ are usually killed. As

[1] Loeb and Northrop (4) raised a few flies on aseptic banana, but all flies were sexually sterile. Guyenot (5) also succeeded in raising a small percentage of flies on sterile potato.

[2] An excellent nontransparent medium consists of yeast cake moistened with water.

adult flies usually carry living yeast cells upon them this medium would be difficult to use for work in heredity. A nutrient medium for yeast would best suit the needs of geneticists. Into such a food the adults or pupæ would carry living yeast cells which would ferment the sugars and produce odorous substances which cause oviposition by the female fly. Larvæ on hatching would spread the yeasts throughout the medium, thus increasing growth and alcoholic fermentation which may prevent the development of injurious microorganisms.

Two media might be suggested for this purpose, viz.: Fermented banana agar and Pasteur's nutrient agar. The former may be prepared by adding two cakes of bread yeast separated in 100 c.c. of water to one dozen mashed bananas, mixing thoroughly and allowing to ferment for twenty-four hours. This material should be pressed through a sugar sack and the liquid resulting thickened by heating with 1.5 gm. agar-agar per 100 c.c. and poured into slant culture tubes, plugged and sterilized.

A perfect nutrient medium for yeast consists of 10 gm. ammonium tartrate, 10 gm. ashes of yeast, 100 gm. rock candy, 1,000 gm. distilled water. This is called Pasteur's culture fluid and can be treated in the same manner as the above but should be sterilized in an Arnold sterilizer for three successive days, as it can not be heated to as high a temperature as the banana without preventing jellation. Pasteur's nutrient solution can be approximated by diluting molasses with three parts of water.

In the two media described above yeasts develop rapidly and furnish abundant food for *Drosophila* larvæ and also produce odors of fermentation which cause the female to oviposit readily. A concentrated food for yeast such as banana flour would probably increase the value of the first medium as this substance is now used for raising yeast in the brewing industry (6).

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